Brief report

Survey of Frequency of bla^{per} Gene in *P. aeruginosa* by Kirby Bauer& PCR Method

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Abstract

Aim: The present study was aimed to investigate frequency of bla per gene in hospital isolates of P. aeruginosa.

Methods: The number of 100 isolates of P. aeruginosa were isolated from in burn Hospital in Tehran during a 1-year study in 2013-2014.The identification were carried out by colonial morphology, pigment formation, positive Oxidase test,; growth test at 42ºC on Nutrient agar, OF, Arginine dehyrolase and Motility tests.
Results: The results indicated that 15% of burned hospital isolates contain bla pe gene. This study indicated that zone diameter mean of growth inhibition ≤ 14 mm to ceftazidime.The rate of MIC to ceftazidime was 20 µg/ml.
Conclusion: According to results of current research we hope in future be used drugs to the clinic with a wider range as a complementary therapy and also for burned infections.
Keywords: *Pseudomonas aeruginosa, Multidrug-Resistant, , bla^{per} , PCR.*

Introduction

Pseudomonas aeruginosa is a Gram-negative non-fermentative bacili belonging to the family Pseudomonadaceae that recently it has gained increasing attention as a nosocomial pathogen (1). These organisms have been implicated in a diverse range of infections (respiratory tract, bloodstream, skin and soft tissue, prosthetic devices) and are a particular problem in intensive care units where numerous outbreaks have been extremely difficult to control. The rapid emergence and global dissemination of P. aeruginosa as major nosocomial pathogen is remarkable and demonstrates its successful adaptation to the 21st century hospital environment. (2, 3). P. aeruginosa is often resistant to a wide variety of antimicrobial agents. Ceftazidime resistance in P. aeruginosa is due to a variety of combined mechanisms such as hydrolysis by beta-lactamases, alterations in the outer membrane protein and penicillin-binding proteins and increased activity of efflux pumps (4).
For many years, control of bacterial infections by inhibiting microbial growth has been a primary approach
of antimicrobial chemotherapy(5). An emerging problem associated with continual indiscriminant use of this therapeutic strategy is the selection of resistant bacteria with higher levels of tolerance against broad-spectrum antibiotics.Development of novel antibiotics that interfere with metabolism coupled with continued indiscriminant use of antibiotics will only lead to evolution of new resistance mechanisms and pathways by bacteria (6). Recently, it has been recognized that there is a need for a strategy that can block very basic mechanisms of bacterial communication that appear to control bacterial virulence factors leading to pathogen city(7). Emerging research has suggested that functions including swarming, biofilm formation, secretion of virulence factors and acquiring competency play an important role in successful and recurrent establishment of bacterial infections in living systems (8).
Pseudomonas spp and possible transition between living and non-living things and as well as long-term survival in the hospital environment enhance the appearance of the bacteria in the hospital environment and infection due to increasing (9). Use of incorrect of medicinal drugs was redounded to distribution of drug resistant genes. With the increase in population and urban growth, and increased use of synthetic drugs, many of these problems of synthetic drugs such as the increasing of antibiotic widespread

resistance emerge among microorganisms and economical detriments was induced people tend to other treatments(10).

Objective

The present study was aimed to investigate frequency of bla ^{per} gene in hospital isolates of *P. aeruginosa***.**

Material and Methods

A retrospective study depends on the registered files of the admitted patients to Prince Ali Bin Alhussein hospital with ACS since April 2013 till October of 2013 included 174 patients.

Results

Isolation of *Pseudomonas aeruginosa*

The number of 100 strain of P. aeruginosa were isolated from in burn Hospital in Tehran during a 1-year study in 2013-2014.The identification were carried out by colonial morphology, pigment formation, positive Oxidase test,; growth test at 42ºC on Nutrient agar, OF, Arginin dehyrolase and motility tests.

Antimicrobial Susceptibility Test

Susceptibility to various classes of antibiotics was determined by the Disc diffusion method in accordance with Clinical Laboratory Standard Institute 2013 (CLSI) guidelines [11]. The testing antibiotics were performed with amikacin (30 µg), Tobramycin (10µg), Gentamaicin(10µg), ciprofloxacin (5µg) and Polymixin B (300unit) and Ceftazidime (30 µg) disks.

Detection of bla ^{per} by PCR

Genomic bacterial DNA was extracted from 100 strains by boiling a suspension of bacteria to 95°C for 5 min in a final volume of 25 µL of distilled sterile water. After centrifugation at 13 000g, the supernatants were used as DNA templates.

PCR was performed in a standard enzyme Taq DNA polymerase. Designed primers with genscript software for amplify target fragment were bla per Forward (5′- ATGAATGTCATTATAAAAGC<C>-3′) Reverse (5′ - AATTTGGGCTTAGGGCAGAA<G>-3′),

The PCR reactions were performed in a final volume of 25 µL containing 12.5 µL Master Mix(1x) and 1 µL of DNA extract(20 pg) , 1 µL F Primer(0.1 - 1µM) , 1 µL R Primer(0.1 - 1µM), 9.5 µL Sterile Deionized Water with Cinnagen kit. The cycles for bla per gene mixtures were incubated 180 s primary denaturation at 95°C, secondary denaturation for 30 s at 95°C, annealing for 40 s at 46°C and extension 30 s at 72°C, followed by a final extension for 90 s at 72°C. that 35cycle was performed. The amplified products were analyzed by electrophoresis on 1% agarose gel (Cinnagen) containing 0.1 g of ethidium bromide per ml in TAE buffer. The PCR product was visualized under UV light and photographed.

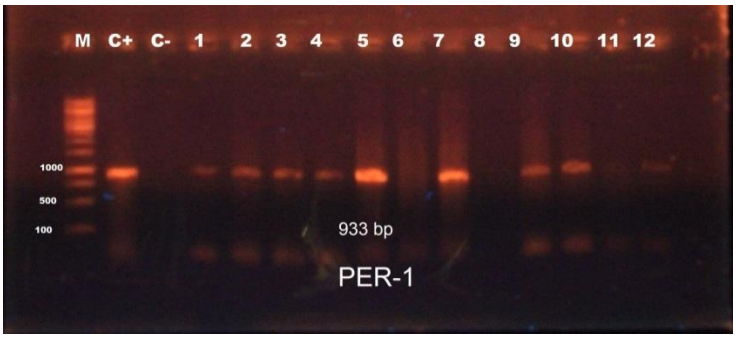
Final extension step of 5 min at 72°C both PCR products were detected on a 1% agarose gel.

Antimicrobial susceptibility testing

Antibiotic susceptibility test results the number of handered P. aeruginosa is as follows. Amikacin (87%) , Tobramycin (83%), Gentamicin (83%), Ceftazidime (85%) and polymixin B (100%), ciprofloxacin (93%) were resistant(table 1) . Inhibitory effects of Results MIC (µg/ml) antibiotics disk of CP and IPM have been indicated in table 2.

Table1: Antimicrobial Susceptibility Pattern to P. aeruginosa Strains

	Resistance (%)
Ceftazidime	(85%)
Tobramycin	(83%)
Gentamicin	(83%)
Amikacin	(87%)
Ciprofloxacin	(93%)
PolymixinB	(0%)



Figuure1: Results of PCR test of bla^{per} gene for *P. aeruginosa* isolates

Line M: marker 1 kb, C^+: positive control, C^-: negative control, Lines 1 to 12 test samples

Table 2: Results of MIC test for Antibiotices

Sample1	Sample 9	P. aeruginosa PAO1	
>4096	>4096	4096	Polymyxin E (µg/ml)
16	32	0.5	CP (µg/ml)
32	16	4	IPM (µg/ml)

Discussion

Nosocomial infections caused by Multidrug resistant strains of *P. aeruginosa* are currently among the most difficult to treatment, and they continue to present serious challenges to clinicians' empirical and therapeutic decisions in burned patient .Outbreaks of extensively, and pan drug-resistant *P. aeruginosa* (XDR, and PDR, respectively) currently as been Enrollment in local colleges, 2005 reported from worldwide(11). In this study, we detect 15% isolates contain bla ^{per} genes from burned patients. Increasing prevalence of XDR *P. aeruginosa* strains and limited treatment options has prompted the use of antibiotics combinations like tigecycline and colistin as therapeutic regimens (12).

Pseudomonas aeruginosa is an opportunistic pathogen that can cause severe hospital-acquired infections, especially in immunocompromised hosts. P.aeruginosa is serious problem for its resistance to antibiotics. Pathogenic microorganisms have to face hostile e According to results of current research we hope in future be used appropriate drugs to the clinic with a wider range as a situation therapy and also for burned patients.

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